Association of the DBH polymorphism rs3025343 with smoking cessation in a large population-based sample

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DBH-geeni koodaa dopamiinia hajottavaa entsyymiä, joka on liitetty palkkiojärjestelmään vaikuttamisen kautta erilaisiin riippuvuuksiin. DBH:n variaatioiden on todistettu liittyvän tupakointikäytänteisiin ja –tapoihin monessa tutkimuksessa. Viimeisimpänä rs3025343 liitettiin tupakoinnin lopettamiseen suuressa GWAS meta-analyysissa. Tutkimuksemme tavoitteena olikin replikoida kyseinen löydös suuressa väestötutkimusnäytteessämme. Lisäksi halusimme tutkia, vaikuttaisiko rs3025343 jonkin muun ympäristötekijän kautta vai itsenäisesti tupakoinnin lopettamiseen.

Tutkimusnäytteemme on peräisin suomalaisesta väestönäytteestä, FINRISK-tutkimuksesta. Siihen lukeutuu 26,582 genotyypattua henkilöä tupakointistatuksineen. Analyysit rajasimme 11,926 yksilöön, jotka olivat joko nykyisiä tupakoitsijoita (n=6,578) tai vähintään 6kk sitten tupakoinnin lopettaneita (n=5,348). Henkilöistä oli saatavilla myös kattavasti muita tietoja mukaan lukien sosioekonominen status, terveyteen liittyviä tapoja ja terveydentila, joita käytimme analyyseissä hyväksi.

Yhteys rs3025343 ja tupakoinnin lopettamisen välillä (OR=1.12, p=0.094, 95%CI=0.98-1.30) osoittautui tutkimuksessamme identtiseksi GWAS-tutkimuksen kanssa (OR=1.12, 95%CI=1.08-1.18). Mikään testatuista fenotyypeistämme ei vaikuttanut tuohon yhteyteen merkitsevästi. Siviilisääty, koulutustaso, masennus, alkoholin käyttö, itseraportoitu terveys sekä COPD assosioituivat fenotyyppitekijöistä tupakoinnin lopettamiseen, mutta mikään edellämainituista assosiaatioista ei riippunut tutkimastamme genotyypistä.

Vaikka tutkimustuloksemme ei ole tilastollisesti merkitsevä, efektikoko viittaa vahvasti siihen, että tutkimallamme polymorfismilla on jonkinasteinen yhteys tupakoinnin lopettamiseen. Merkitevyyden esiinsaamiseksi riittävällä voimalla (80%) otoskoon tulisi olla niinkin suuri kuin 36,092 tapausta ja 29,343 kontrollia, koska harvinaisemman alleelin kantajia on suhteellisen vähän (7,1%). DBH-geenin variaatiot ovat osoittautuneet monessa tutkimuksessa olevan yhteydessä nikotiiniriippuvuuteen tai tupakoinnin lopettamiseen. Jos näitä yhteyksiä saadaan tutkittua lisää, on mahdollista että tietoja voitaisiin käyttää hyväksi tulevaisuudessa esim. yksilöllisesti räätälöidyssä tupakoinnin lopettamisen hoidossa.

Avainsanat – Nyckelord – Keywords

Smoking cessation, nicotine dependence, genetics

Säilytyspaikka – Förvaringställe – Where deposited

Muita tietoja – Övriga uppgifter – Additional information

Abstract

Introduction

Genetic variations in *DBH*-gene and its surroundings have been shown to associate with smoking behavior including smoking cessation in several studies. In this study we replicate and measure the effect size for association between *DBH* polymorphism rs3025343 and smoking cessation in a large population-based sample while examining environmental factors that could relate to the association.

Methods

We studied 11,926 adult subjects from four surveys of the National FINRISK Study. The analysis was restricted to either current or former smokers. Logistic and linear regression analyses were conducted to investigate the relationships of the SNP, covariates, smoking cessation and smoking severity (cotinine, CPD). Gene-environment interactions were tested by likelihood-ratio test.

Results

The association between rs3025343 and smoking cessation (prevalence odds ratio, OR=1.12, p=0.094, 95%CI=0.98-1.30) was replicated identically with the GWAS study of The Tobacco and Genetics Consortium (OR=1.12, 95%CI=1.08-1.18). None of our tested phenotypes significantly influenced the association between rs3025343 and smoking cessation. Overall, marital status, education, depression, alcohol use, self-rated health and COPD showed phenotypic associations with smoking cessation, but the association of various phenotypes with smoking cessation did not vary by genotype.

Conclusions

The current study replicates the effect size for the association between rs3025343 and smoking cessation despite lack of overall significance due to smaller sample size. We could not show environmental influences on the association of rs3025343 with smoking cessation.

Implications

Our study replicates the direction and strength of the association of DBH polymorphism rs3025343 with smoking cessation. We could not detect environmental influences on the strength of the association of rs3025343 with smoking cessation, but the limited power of our analysis needs to be taken into account.

Introduction

Quitting smoking is followed by immediate health benefits and a reduced risk of morbidity and mortality. Of the smokers that quit on their own only as few as 4-7% do not lapse back to smoking within the first year [1]. Even when utilizing cessation treatments, only 15-25% maintain abstinence for at least one year [2]. A better understanding of the genetic background of smoking cessation may provide the means to develop new and more efficient treatments, especially when the environmental factors are also taken into consideration. Heritable influences have been found for the ability to quit smoking [3-6]. However, the evidence linking the best known single genetic variants to smoking cessation has been conflicting in observation studies and clinical trials [7-9].

Nicotine enhances dopamine release by activating dopaminergic neurons in the mesolimbic reward pathway, which is involved in feeling pleasure when smoking. The *DBH*-gene encodes dopamine β-hydroxylase, which catalyzes the conversion of dopamine to norepinephrine, and is also associated with smoking and drug dependencies.

The SNP rs3025343, located 23kb upstream of *DBH*, is associated with smoking cessation according to a very large GWAS meta-analysis. The study, which included 64,924 ever smokers (OR=1.12, 95%CI=1.08-1.18) found the G allele of rs3025343 to be more common among former than current smokers [10]. Another study with 3,441 chronic obstructive pulmonary disease (COPD) patients of European ancestry replicated this association by demonstrating similar results (OR=1.24, p=0.015) [11]. Both studies were observational.

In a pooled analysis of two clinical trials reported by Leventhal and colleagues a haplotype of 6 *DBH* SNPs was found to predict abstinence at the end of cessation treatment and 6-month follow-up in study samples with high nicotine dependence [12]. Another study reported one DBH polymorphism to be associated with smoking severity among male schizophrenic smokers [13]. Although distinct from SNPs found significant in GWA studies, these findings support the hypothesis that *DBH* is an important candidate gene involved in smoking behavior.

Our aim was to replicate the result of GWAS regarding rs3025343 and its association with smoking cessation in a large population-based sample. Furthermore, analyses of the relationship between rs3025343 and environmental factors associated with smoking cessation were conducted in order to observe the influence of such environmental factors on the strength of the association of rs3025343 with smoking cessation. Analyses of per-allele associations were conducted using a random effects method.

Materials and methods

Study sample

Our sample was drawn from a Finnish population-based survey, the National FINRISK Study, which was first initiated in 1972 and has since then been carried out every five years using independent samples from four to six different parts of Finland depending on the year of survey [14]. We used data from cohorts 1992, 1997, 2002 and 2007, comprising of 26,582 genotyped persons with known smoking status. Our analysis focused on the 11,926 genotyped subjects (41% women) who were either current smokers (n=6,578) or had quit smoking at least six months prior to the survey (n=5,348). Other self-reported information about their health-related habits, socioeconomic status and health status were obtained (see supplementary material for details on traits and genotyping). The mean age was 47.7 years (SD 12.7, range 25-74).

Statistical analyses

For testing the associations between covariates and quitting smoking within different genotypes we combined AA and AG genotypes to get two genotype classes; AX & GG. This was done due to AA genotype being too rare to permit separate analyses as is visualized in **Table 1**. We conducted logistic and linear regression analyses to investigate the relationships of the SNP, covariates, smoking cessation and smoking severity. To examine whether the associations of the gene with outcome differed significantly by categories of covariates, we fit models with and without an interaction of the SNP and covariate, and tested the

significance of the change in model fit using a likelihood ratio test to derive the significance of the interaction. Age, gender, region and the year of questionnaire were adjusted in the analysis.

All statistical analyses were performed using Stata 13.1 [15]. We report nominal p-values throughout the manuscript.

Results

The effect size (OR=1.12, p=0.094, 95%CI=0.98-1.3) for association between rs3025343 and smoking cessation in the FINRISK study sample was replicated identically with the earlier GWA study (OR=1.12, 95%CI=1.08-1.18) although the association did not yield statistical significance [10]. This is shown in **Table 1** together with genotype information of the study sample. Rs3025343 G allele was more abundant in Eastern Finland (North-Karelia & Northern Savo) than elsewhere. Allele frequencies were similar regardless of the survey year.

Table 2 shows the results of the association analysis between smoking cessation and rs3025343 in different phenotypes. While variation in OR-values was observed within phenotype classes, the likelihood-ratio tests show that none of the gene-environment interactions exceeded statistical significance ($p<0.05$). Thus, it appears that in this study, none of our tested phenotypes affects overall the strength of the association between rs3025343 and smoking cessation. Within variables, some individual categories showed nominally significant associations: among married persons (OR=1.18, 95%CI 1.00 to 1.39), among those drinking 14 or more drinks weekly (OR=1.60, 95%CI 1.01 to 2.52) and among those living the most eastern province of Finland (OR=1.52, 95%CI 1.05 to 2.21). After adjustment for multiple testing, none of these is significant.

The logistic regression analysis of the rs3025343 genotypes on smoking cessation in different phenotypes examined the strength of the association between phenotype and smoking cessation within the main genotype classes (first two columns of results) and overall (last column) **(Supplementary table 1)**. The associations of phenotypes with smoking cessation were very similar in both genotype groups.

However, **Supplementary table 1** shows certain interesting results independent of genotype. Overall, having a diagnosis of bronchial asthma (OR=1.19, 95%CI=0.99-1.43) seems to make quitting smoking a little more probable while having COPD (OR=0.55, 95%CI=0.46-0.65) influences vice versa. Not surprisingly, quitting smoking is more likely for married people than singles, divorced or widowed. The same applies for high educated over low-educated people. Also, the better the self-rated health is, the greater the likelihood of having quit smoking.

Quitting was more likely for people who have never used medication for depression than for those who have **(Supplementary table 1)**. Expectedly, alcohol consumption was also strongly related to smoking cessation; greater the alcohol use, the smaller the likelihood of having quit smoking. The effect is best seen in heavy drinkers (OR=0.48) while abstainers comprise the reference group.

The rs3025343 allele did not affect cotinine levels (p=0.78) nor CPD significantly (p=0.21) among current smokers, i.e we find no influence of the variant on amount of smoking.

Discussion

Several studies have reported association between common variants and smoking cessation although most studies have shown only modest associations. Nevertheless, a large meta-analysis has shown significant association between one single nucleotide polymorphism, rs3025343 near *DBH* gene, and smoking cessation [10] and a second large meta-analysis, in African Americans (David, 2012, PMC3365260), showed nominal significance between rs3025343 and smoking cessation. This finding has been replicated among COPD-patients [11]. Moreover, a candidate gene study reported rs3025343 to be nominally associated with continuous FTND score in European Americans (p=0.023) [16]. Based on present knowledge, FTND is one of the strongest predictors of smoking cessation. This indicates that rs3025343 may have an influence on the level of nicotine dependence and further, on smoking cessation [16]. Our study aimed to replicate the effect size for association between rs3025343 and smoking cessation that was reported in the meta-analysis of the Tobacco and Genetics Consortium. We also evaluated the roles and associations of different

environmental factors in order to exclude their possible influence on the relationship between the SNP and smoking cessation. The effect size for the association between rs3025343 and smoking cessation was identical to that observed in the meta-analysis (OR=1.12, p=0.094, 95%CI=0.98-1.3) although the association was not statistically significant in our study.

In addition to studies investigating association between rs3025343 and smoking cessation, diverse studies have been carried out about other polymorphisms near or within *DBH*-gene and their relation to smoking cessation, usually through nicotine dependence level [17-21]. Although their results appear somewhat inconsistent, these studies demonstrate the potential role of *DBH* in smoking-related behaviors. The key limitation of many studies concerning *DBH* and smoking cessation and main reason for insignificant results is small sample size since even modest genetic influences usually require large study samples to be detected. Many polymorphisms of *DBH* associated with smoking cessation may be in strong linkage disequilibrium with each other.

Rs3025343 does not cause a change in the amino acid residue in DBH since it is located in an intergenic region. Nevertheless, it could modify gene expression by affecting transcription either directly, through or together with other genetic variants in strong LD. The fact that only one DBH upstream variant (rs1611115) causes 51% of the variation in plasma-DBH activity in European-Americans strongly supports the conception that intergenic variants have a notable role in the regulation of gene expression [22].

Despite our relatively large total study sample size, minor allele carriers are rather few (7.1%). Thus, especially when inspecting disease phenotypes, the number of minor allele carriers becomes very small when examining different phenotypes within genotypes. This leads to the statistical power being too low (22% according to the power analysis) for demonstrating reliable conclusions regarding minor or major allele's specific influences on smoking cessation. This is the main limitation of our study. To increase power to 80%, as many as 36,092 cases and 29,343 controls would be required in total due to the low minor allele frequency. It should also be taken into account that we inspected and ruled out only a few carefully pre-selected covariates.

It is of interest that the *DBH* SNP has a different allelic distribution within Finland. The most probable explanation for the deviating allelic distribution is that the gene pool of people in Eastern and Western Finland are subject to influence from different regions together with the relatively small and regionally immobile population**.** Also, the role of that G allele on smoking cessation appeared to be more significant in North-Karelia (OR=1.52, 95%CI= 1.05-2.21) than elsewhere, which can partly be due to the different allelic distribution. The regional distribution however did not affect our other results, as we adjusted for region in all analyses.

The current study replicates the effect size for the association between rs3025343 and smoking cessation although the association was not statistically significant. In addition we document associations of several covariates with smoking cessation.

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Declaration of Interests

Tellervo Korhonen has consulted for Pfizer Finland on nicotine dependence in 2011-2016. Jaakko Kaprio has consulted for Pfizer Finland on nicotine dependence in 2012-2015.

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Supplementary materials

1. Phenotypes

Smoking-related traits

Smoking status was determined by three questions: “Have you ever smoked regularly, almost every day for at least a year?”, “Do you smoke now?” and “When was the last time you smoked?”. Based on this information, participants were divided into categories of daily smokers (smoked today or at least yesterday), occasional smokers (smoked two days to a month ago), recent quitters (quit one to six months ago) former smokers (quit one month ago or earlier) and never smokers (never smoked regularly).

In the analyses of smoking cessation associations we used the groups of current smokers (comprising daily smokers above) and former smokers. Since relapse to smoking is highest within the 6 months of quitting smoking, recent quitters, i.e. smokers who had quit smoking less than 6 months prior to the survey (1.9% overall) were excluded from the analysis. Occasional smokers (1.6% overall) were also excluded from the analysis. Vartiainen et al. have shown that smoking status is reported accurately when assessed by cotinine analysis using these questions and definitions [1].

Participants were asked: “How many cigarettes (manufactured or self-rolled cigarettes, or equivalent amounts of cigars or pipe tobacco) do you smoke daily or smoked before quitting?” to measure cigarettes per day (CPD) amount. The answers were adapted as a quantitative measure of CPD that was analyzed as a quantitative trait when examining the association between CPD and our candidate SNP.

Other traits

We selected other traits for inclusion in the analysis based on their availability in the FINRISK database and their relevance for smoking cessation. These come from three domains of traits: alcohol use, smoking related disease and sociodemographic characteristics.

Alcohol consumption was defined as two different traits for the analyses. First trait was categorical and the other dichotomous. Participants were asked: “How many glasses (restaurant measures) or bottles of alcohol beverages did you drink during last week?”. Based on this, alcohol consumption (g/week) was calculated by means of knowing ethanol content (grams) of each alcohol beverage. For categorical alcohol use analyses the quantitative alcohol consumption was first coded as standard drinks per week; one alcohol drink was defined as containing 12g of pure ethanol. After that, individuals were categorized as abstainers (less than 1 drink per week), light drinkers (3-7 drinks per week), moderate drinkers (7-14 drinks per week) and heavy drinkers (>14 drinks per week). For dichotomous alcohol use, we contrasted those drinking one or more drinks per week versus those either using no alcohol at all or drinking less than one drink per week.

Information about certain smoking-related diseases; Angina pectoris, COPD and bronchial asthma, of participants was asked. They were asked if they have had these diseases (diagnosed by a physician) during last 12 months.

Educational level was classified as low, middle and high by dividing self-reported years of formal education into study-year and cohort specific tertiles. Two most recent questionnaires also included the question “What is your highest educational level?” with seven different response options; (1) elementary school, (2) middle school, (3) trade school, (4) high school (5) vocational school (6) college and (7) academic degree.

Marital status was asked by “What is your marital status” with alternatives of (1) Married, (2) Cohabitation, (3) Single, (4) Divorced and (5) Widowed. Married and cohabitation groups were combined as their influences on smoking cessation can be seen similar, while the latter three were combined as persons without regular partners.

The subjects were asked about their self-rated health using the alternatives: (1) very good, (2) good, (3) average, (4) rather poor, and (5) very poor.

Depression among participants was determined by question “When is the last time you have used medication for depression?” with alternatives: (1) During the past week, (2) 1–4 weeks ago, (3) 1–12 months ago, (4) Over a year ago and (5) Never. This definition limits depression to the diagnosed form of it,

which has the advantage that diagnosed cases are often more reliable than self-rated ones. On the other hand, many cases of depression are never diagnosed or have sought treatment. Thus the prevalence of depression in our analyses is lower than in reality.

2. Analyses

Age was used in the analyses as a continuous variable. In addition to that, gender was also adjusted.

Region was one of the adjusted covariates in the analyses. FINRISK surveys are conducted in 6 different study areas: 1) North-Karelia province, 2) Northern Savo province, 3) cities of Turku and Loimaa, 4) cities of Helsinki and Vantaa, 5) Oulu province and 6) Lapland. Region was adjusted for in all analyses because of different allelic distribution of rs3025343 within Finland.

The study year was also adjusted in analyses since the number of participants was dependent of the year and common knowledge and conceptions of smoking may have changed during twenty years. Allelic distribution was independent of the study year.

3. Genotyping

The SNP of our interest, rs3025343 was selected based on the meta-analysis of the Tobacco and Genetics Consortium [2] and supported by the study of Siedlinski and colleagues, which replicated the finding [3]. A second large meta-analysis, in African Americans (David, 2012, PMC3365260), showed nominal significance between rs3025343 and smoking cessation as well.

DNA was derived from whole blood samples frozen immediately at the clinical study sites. DNA was extracted at the National Institute of Health and Welfare. Genotyping of the DNA samples was performed as described previously [4]. The success rate of rs3025343 was >0.99 and it was in Hardy Weinberg Equilibrium ($p=0.87$). Overall minor allele (A) frequency was 0.036.

Plasma cotinine level was measured by gas chromatography from fasting plasma samples for participants who had reported to smoke currently [5]. We received both plasma cotinine level and genotype information of rs3025343 from 1495 current smokers in total from the 1992 survey.

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Table 1. Genotype details of study sample and association between smoking cessation and rs3025343 per allele in Finnish population sample (logistic regression analysis), number of participants in different genotypes, p-value, odds ratio (OR) per allele and 95% confidence interval (95% CI) in columns.

	AA	AG	GG	total	p	OR	95% CI
FINRISK							
Former	6	351	4,991	5,348			
Current	9	482	6,087	6,578			
All	15	833	11,078	**11,926**	0.094	1.12	0.98, 1.29

Table 2. Association between smoking cessation and rs3025343 by strata of different phenotypes in the FINRISK population sample, prevalence odds ratios (ORs) and 95% confidence intervals (95% CIs) from logistic regression. Gene-environment interactions; p-value for interaction (p_i) from likelihood-ratio test.

Phenotype	OR	95% CI	p_i	n	%
Sex			0.50	11,926	100
Male	1.10	0.91, 1.33		7,030	59
Female	1.16	0.93, 1.45		4,896	41
Marital status			0.62	11,909	100
Married	1.18	1.00, 1.39		8,749	73
Single	0.89	0.59, 1.36		1,533	13
Divorced	1.08	0.67, 1.75		1,334	11
Widowed	1.26	0.44, 3.63		293	2
Education			0.19	11,806	99
Low	1.14	0.89, 1.47		4,260	36
Medium	1.28	0.99, 1.64		4,005	34
High	0.94	0.73, 1.22		3,541	30
Region				11,926	100
North-Karelia	1.52	1.05, 2.21		2,321	19
Northern Savo	1.15	0.80, 1.66		2,313	19
Turku-Loimaa	1.14	0.85, 1.52		2,275	19
Helsinki-Vantaa	1.11	0.83, 1.49		2,558	21
Oulu	0.91	0,61, 1.35		1,648	14
Lapland	0.78	0.45, 1.37		811	7
Angina pectoris			0.68	11,825	99
No	1.12	0.97, 1.30		11,295	95
Yes	1.08	0.52, 2.19		530	4
Bronchial astma			0.40	11,814	99
No	1.14	0.99, 1.33		11,293	95
Yes	0.80	0.42, 1.55		521	4
COPD			0.63	11,818	99
No	1.14	0.98, 1.32		11,157	94
Yes	0.82	0.40, 1.67		661	6
Self-rated health			0.83	11,845	99
Very good	0.98	0.65, 1.47		1,330	11
Good	1.19	0.95, 1.49		4,932	41
Average	1.18	0.93, 1.50		4,402	37
Rather poor	0.97	0.58, 1.62		1,083	9
Very poor	0.70	0.15, 3.27		98	1
Alcohol use during last week				11,926	100
Yes	1.14	0.96, 1.35		8,441	71
No	1.11	0.87, 1.44		3,485	29
Alcohol use (drinks/week)			0.75	11,660	98
abstainers	1.11	0.86, 1.44		3,485	29
>0-3	1.10	0.78, 1.53		2,024	17
>3-7	1.10	0.78, 1.54		2,289	19
>7-14	1.15	0.81, 1.64		2,171	18
>14	1.60	1.01, 2.52		1,691	14